DOUBLE FERTILIZATION IN COMPOSITAE.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXI.

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(WITH PLATES XV AND XVI)

IN August 1898, Nawaschin communicated to the Russian Scientific Congress at Kieff the results of his work on fertilization in *Lilium Martagon* and *Fritillaria tenella*. Guignard, upon learning of the discoveries of Nawaschin, contributed to the Academie des Sciences a short account of his unpublished researches upon fertilization in several species of Lilium. Miss Sargant, from a reexamination of her preparations of *Lilium Martagon*, fully confirmed the observations of both Nawaschin and Guignard. A recent study of *Tulipa sylvestris* and *T. Celsiana* by Guignard gives results in strict accord with the earlier observations.

These observers find that both male cells upon emerging from the pollen tube are vermiform and twisted on their axes, suggesting the idea of non-ciliated spermatozoids. One male cell, coming in contact with the egg nucleus, retains for a time its vermiform shape, gradually enlarges until it becomes nearly spherical, and finally fuses with the egg nucleus. The other male cell fuses with the upper polar nucleus, and the nucleus resulting from this fusion unites with the lower polar nucleus. Sometimes the polar nuclei fuse and then unite with the male cell, and sometimes the polar nuclei and the male cell fuse simultaneously. Preparations of *Lilium Philadelphicum* and *L. tigrinum* in this laboratory show the last named condition. Miss Sargant figures one case in which the ends of the male cell are applied to the polar nuclei, uniting them as if by a bridge. Experiments in hybridization by De Vries and Correns seem to

indicate that double fertilization may be more frequent than is commonly supposed.

So far as recent work indicates, the spermatophytes produce two male cells. The persistent appearance of a second male cell, seemingly as well organized as the one which functions, has found no better explanation than a phylogenetic one, although it would be hard to explain why a cell which has long been abandoned continues to be well organized. We may well inquire whether a simultaneous fertilization of the egg and of the endo sperm nucleus may not be universal in angiosperms.

The study of the mature embryo-sac of Erigeron and of Silphium was undertaken for the purpose of determining the fate of the second male cell. The first named genus was chosen because a large number of ovules in different stages of development could be cut at once; the second, because of the differentiation shown by the disk and ray flowers.

MATERIAL AND METHODS.

Material was collected in the vicinity of Chicago, from June 25 to July 20, 1900. Collections were made at all hours of the day, and all material was killed in the field. The outer involucral scales of Erigeron were removed and the heads closely trimmed, only enough of the receptacle being left to hold the ovules together. A 1 per cent. aqueous solution of chromo-acetic acid was used as a killing and fixing agent. Carnoy's fluid was found to be unsatisfactory. The material was passed through xylol into paraffin and cut in serial sections 3.3 μ and 6.6 μ thick.

The method of treating Silphium was slightly different from that for Erigeron. The ovules, except those intended for tracing the path of the pollen tube, were freed from the surrounding tissues and immediately plunged into chromo-acetic acid at a temperature of about 100°, and were allowed to remain in the hot acid about two hours. After washing and dehydrating, they were passed through xylol into paraffin at a temperature of 63°. Sections 2–5 μ thick were cut on a Reichert rocking microtome.

Flemming's safranin-gentian-violet-orange G and Haidenhain's iron-alum-haematoxylin both gave excellent results, but the most satisfactory differentiation in fertilization stages was obtained with cyanin and erythrosin. This last combination, after the sections had been treated with acetic acid and chloroform, gave details of structure not obtained by other methods.

ERIGERON.

Two species, *E. Philadelphicus* L. and *E. strigosus* Muhl., were examined. The following statements apply to both, as there is no difference in their embryo sacs, except in size. The development of the embryo sac does not differ essentially from that of other Compositae. The number of antipodal cells varies, and the one nearest the endosperm nucleus is frequently binucleate.

The lower polar nucleus moves up and unites with the upper polar nucleus (*fig.* 1), which remains near the egg. The nucleoli are very conspicuous, with highly refractive bodies scattered through them.

The mature egg is pear shaped, its nucleus being very large (*fig.* 2) and showing a fine network of chromatin. A vacuole near the middle of the egg is very conspicuous.

The synergids are pear shaped, and the position of their nuclei is variable. The end nearest the egg invariably has a large vacuole, while the smaller end stains deeply and sometimes has a distinct constriction (*fig.* 2) between the nucleus and the tip.

The embryo sacs in the ovules upon the edge of the head are larger than those at the center, several cases being observed in which the sacs in the center of the head did not function. In *E. Philadelphicus* the usual length of the embryo sac when ready for fertilization is about 216 μ, the width being about 40 μ. The endosperm nucleus at this stage is from 14.4 to 19.8 μ in diameter, its nucleolus being usually 9 μ. The egg nucleus seldom varies from 10.8 μ, with a nucleolus measuring 5.4 μ. The endosperm nucleus is usually about 16 μ distant from the egg. Chamberlain has shown that in *Aster Novae-Angliae* the nucleolus

of the endosperm nucleus is very constant in size. Many measurements of the egg nucleolus of *E. Philadelphicus* were made, and in only one instance did it vary from 5.4 μ.

The male cells were not observed in the microspores, and no pollen tubes were found except in the embryo sac. When the pollen tube (*pt, fig. 3*) enters the sac, the synergids rapidly disintegrate, so that by the time fertilization is effected only fragments of their nuclei are visible. One male cell fuses with the egg nucleus, and at the time of fusion (*fig. 3*) cannot be distinguished from the egg nucleus. The other male cell fuses with the nucleus which is formed by the union of the polar nuclei, the product of this second fusion being the definitive or endosperm nucleus.

No male cells were observed in the pollen tube, or in the embryo sac before fertilization, so that nothing can be said concerning their previous appearance. The pollen tube, after it has discharged the male cells, usually contains two bodies of irregular shape (*fig. 3*), which stain intensely with cyanin. They were also observed in preparations of Lilium. These bodies may be mistaken easily for male cells, especially after fusion is complete. Nothing could be determined concerning their origin, although it is possible that they may have come from a division of the tube nucleus, since that still remains to be accounted for.

After a brief rest the definitive nucleus divides, and in the many preparations examined the cell plate was invariably parallel to the longer axis of the sac. The endosperm nuclei, after the last named division, are usually multi-nucleolate. The fertilized egg in the meantime shows little change, except a thickening of the wall and a slight enlargement; also it moves down nearer the center of the sac. At this stage scarcely any traces of the synergids can be seen.

In the second division of the endosperm nuclei the cell plate is usually at right angles to the long axis of the sac. The two upper nuclei resulting from this last division move towards the micropylar end of the sac, and, occupying the place made vacant by the synergids, lie a little above and close against the

egg. This last position may have been taken because of a movement of the nuclei in the direction of least resistance, which would be towards the place lately occupied by the synergids.

The fertilized egg usually completes its first division shortly after the second division of the endosperm, the first wall being transverse. The further development of the embryo does not differ from that of the other Compositae which have been investigated.

SILPHIUM.

Silphium integrifolium Michx., *S. terebinthinaceum* L., and *S. laciniatum* L. were studied. The last named species received more attention than the others on account of the large size of the ovules, and the ease with which they could be oriented for sectioning. Merrill's recent paper on the life history of the various species is so complete, except in regard to fertilization, that we will pass over all other stages.

As in Erigeron, the polar nuclei fuse long before fertilization, and the resulting nucleus comes to rest near the egg. A careful study of the microspores of *S. laciniatum* revealed the presence of long male cells, lying side by side and reaching almost around the interior of the spore. The pollen tube usually enters at one side of the synergids. In one preparation it was observed to turn almost at right angles, pass between the nucellar cap and the synergid, and then continue its course towards the egg. The synergid against which the pollen tube lies soon begins to show signs of disintegration, the other usually remaining intact until the fertilized egg begins to divide. In all preparations examined the pollen tube was much expanded above the nucellar cap, and its walls stained intensely.

Fig. 7 shows two small bodies (x) similar to those noted in Erigeron, which remain in the pollen tube after the male cells have been discharged. This figure also shows the coiled male cell (sp_1) resting against the egg nucleus (o). The second male cell (sp_2) is lying against the endosperm nucleus (e). The path by which it reached its destination can be seen

clearly. The cause of the peculiar appearance of the endosperm nucleolus shown in *fig.* 7 is not known, but it is not believed to be due to reagents, as the other structures appear to be normal. *Fig.* 8 also shows the coiled male cells lying against the egg and the endosperm nucleus. *Fig.* 9 shows a more advanced stage of fertilization, the male cell fusing with the egg (*a*), having lost its coiled form. In the other (*b*), a trace of the coil may still be seen faintly. One is closely applied to the egg nucleus, the other to the endosperm nucleus. The irregular shape of the endosperm nucleus shown in *fig.* 7 is also apparent here. Nawaschin, in a paper received since the above mentioned figures were drawn, describes coiled male cells in the embryo sac of *Helianthus annuus* and of *Rudbeckia speciosa*. His description of the reticulated porous structure shows them to be similar to those found in Silphium.

The large amount of food present in the egg is shown in *fig.* 9, the deeply stained bodies being starch grains.

After fusion with the male cell the endosperm nucleus divides rapidly (*ee*, *fig.* 10), and soon the sac is filled with a mass of nuclei. The fertilized egg (*fo*, *fig.* 10) does not divide immediately after fertilization, but rests for some time. It may be of interest to note that the nucleus of the upper antipodal becomes very large at this stage. The appearance of the antipodals suggests that they, for a time at least, may possibly serve to transmit food to the embryo, and also to the endosperm.

Merrill has shown that eight is the characteristic gametophyte number of chromosomes, and he believes that sixteen is the number in the tapetal cells. The writer was unable to make an accurate count, but sixteen appears to be the number in the embryo, and more than sixteen were counted in the endosperm. It is believed, as the result of many counts, that the number in the endosperm is twenty-four. This is the number we should expect here, for the primary endosperm nucleus results from the fusion of three nuclei, each containing eight chromosomes.

SUMMARY AND CONCLUSIONS.

In Erigeron the pollen tube passes down a short distance into the sac and discharges the male cells, which bore their way through the surrounding cytoplasm. One male cell fuses with the egg nucleus, the other with the endosperm nucleus. The endosperm nucleus, after fusion with the male cell, rapidly divides and soon fills the sac with a mass of nuclei. The fertilized egg remains some time in the resting condition, doing little beyond developing a dense membrane and becoming slightly larger. The first wall is transverse, as is usual in angiosperms.

In Silphium the polar nuclei fuse long before fertilization. The pollen tube passes down into the sac and discharges two male cells. These cells, in some instances, leave a well-marked path through the cytoplasm. They retain their coiled form for some time after contact with the nuclei with which they ultimately fuse. One fuses with the egg nucleus, the other with the endosperm nucleus. Before fusion is completed they become nearly spherical, being slightly flattened on one side.

I may venture to suggest that the fusion of the male cell with the endosperm nucleus is a true fertilization, and not a false one ("une sorte de pseudo-fecondation"), as regarded by Guignard. May not the two nuclei—egg nucleus and polar nucleus—nearest the middle of the embryo sac both be considered egg nuclei; and may not one of them, by some means not yet understood, have an advantage over the other, and so develop a more definite structure? May it not be possible that the fusion of three cells — the polar nuclei and the second male cell — to form the endosperm nucleus imparts to that nucleus an excessive stimulus to cell division, resulting in the rapid cell multiplication which immediately follows fusion?

That both the fertilized egg and the endosperm nucleus are potential sporophytes seems a reasonable view, yet we are confronted with the fact of the fusion of the polar nuclei, and until the meaning of this is made clear we must remain in doubt concerning the true significance of double fertilization.

The spermatozoid form of the male cell may be wide-spread in monocotyls and dicotyls. In all the forms recently examined they have been reported as present, either in the pollen tube, in the embryo sac, or lying against the nuclei. In Lilium and Tulipa the male cells seem to assume the vermiform shape in the pollen tube, since in the microspore they are spherical. In Silphium they assume the elongated form in the microspore.

At present double fertilization is known to be a fact in the Liliaceæ. There are indications that it will be found among the orchids. Among the dicotyls it has been found in *Delphinium elatum*, *Helianthus annuus*, and *Rudbeckia speciosa*. It has also been doubtfully suggested in Juglans. The present paper adds to the list *Erigeron Philadelphicus* and *Silphium laciniatum*.

The experiments of De Vries and Correns, and also of Webber, in hybridization of *Zea Mays*, indicate that we may confidently expect double fertilization to be reported soon in that species.

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EXPLANATION OF PLATES XV AND XVI.

All the drawings are made with the aid of a camera lucida, Bausch and Lomb $\frac{1}{12}$ obj. and ocular 1, giving a magnification of 1260. The plates are reduced to one half the original size. A Bausch and Lomb $\frac{1}{16}$ obj. was used for studying minute details.

PLATE XV. Erigeron Philadelphicus.

FIG. 1. Embryo sac with fusing polar nuclei, *e;* synergids, *sy*; egg nucleus, *o*.

FIG. 2. Mature embryo sac showing the long-beaked synergids, *sg;* egg, *o;* endosperm nucleus, *e*.

FIG. 3. Fertilization; pollen tube, *pt*, with two intensely stained bodies, *x;* male cell fusing with the egg, *a;* second male cell fusing with the endosperm nucleus, *b*.

FIG. 4. First division of endosperm.

FIG. 5. Later stage of endosperm division, showing cell plate and multinucleate nuclei.

FIG. 6. First division of fertilized egg.

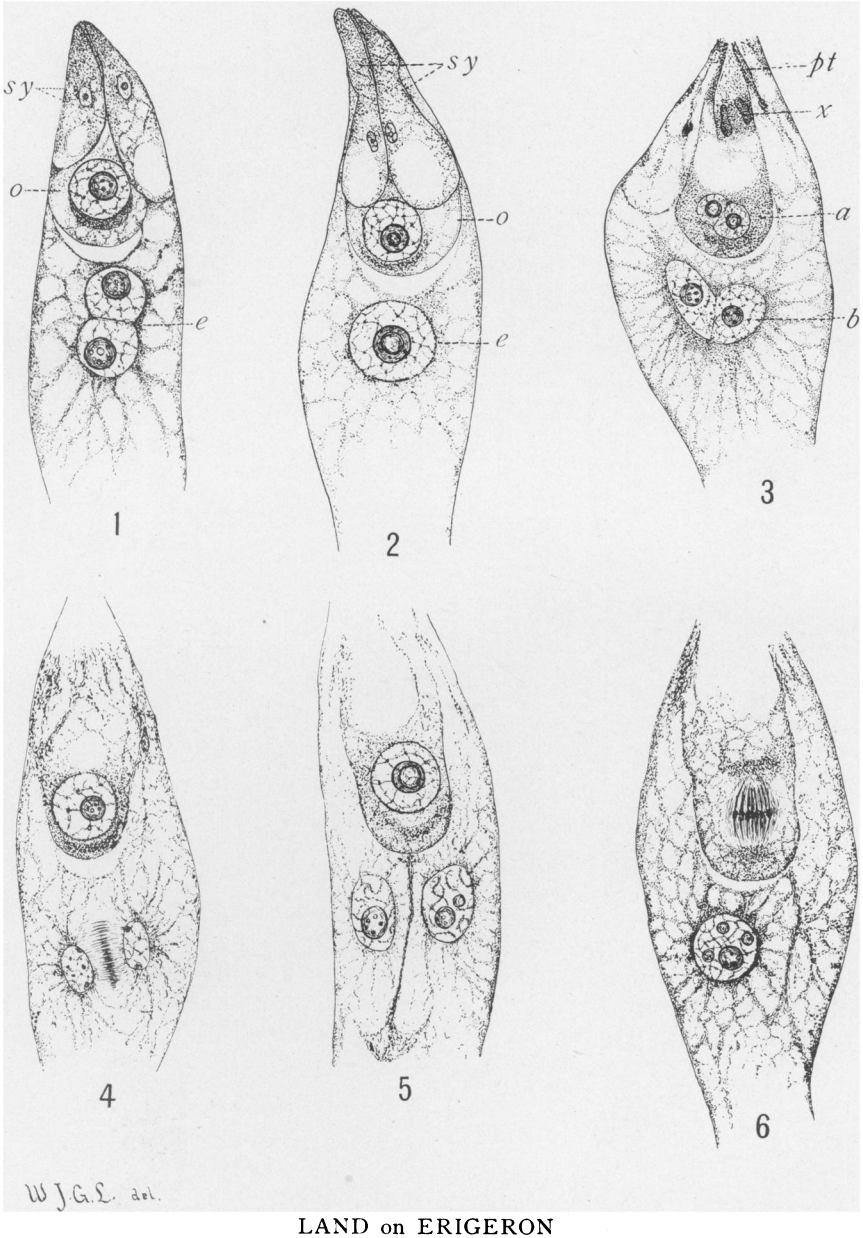
PLATE XVI. Silphium laciniatum.

FIG. 7. Fertilization; pollen tube, *pt*, passing into the sac; synergid, *sy;* unknown bodies, *x;* male cell, sp_1, lying against the nucleus of the egg, *o;* male cell, sp_2, lying against the endosperm nucleus, *e*.

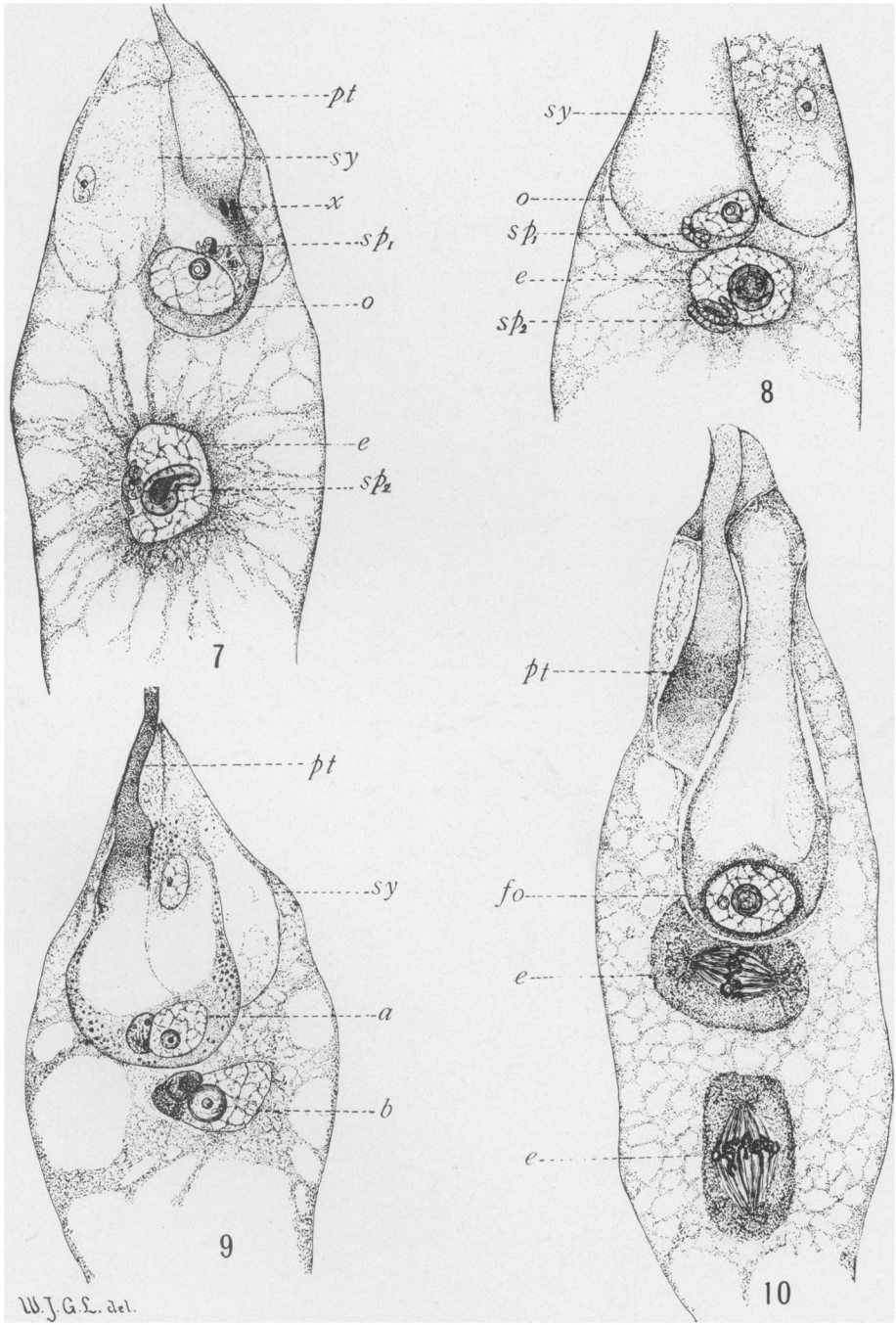
FIG. 8. Synergid, *sy;* male cell, sp_1, lying against nucleus, *o* (a portion of this male cell was lost in sectioning); male cell, sp_2, with large coil, resting against endosperm, *e*.

FIG. 9. Later stage of fertilization; pollen tube *pt;* synergid, *sy;* fusion of male cell with egg nucleus, *a;* fusion of male cell with endosperm nucleus, *b*.

FIG. 10. Second division of endosperm; pollen tube, *pt;* fertilized egg, *fo;* dividing endosperm nuclei, *e*, *e*.



LAND on ERIGERON



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